

Antimicrobial sensitivity pattern of aerobic bacteria isolated from hunting dogs in Maiduguri, Nigeria

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ABSTRACT

Hunting dogs may be a potential source of transmission of pathogenic, zoonotic, or drug-resistant bacteria to people since they may carry the pathogenic bacteria in their oral cavities. As a result of clinic-based cross-sectional study conducted to evaluate the level of multidrug resistant bacteria, colonization of the bacteria in the oral cavities of hunting dogs that appeared apparently healthy were found in Maiduguri. Oral swab samples were taken from one hundred and ninety-three (193) apparently healthy hunting dogs. Using colony morphology and oxidase, catalase, starch hydrolysis, casein hydrolysis, indole, methyl-red and Voges-Proskauer test (MR-VP) the bacteria were isolated and identified. The isolates were tested for antibiotic susceptibility to ten (10) different antibiotics using the Kirby-Bauer disk diffusion method in accordance with standard procedure. *Escherichia coli* (*E. coli*) accounted for the greater proportion of 100 (51.8%) of the isolates. Others were *Staphylococcus* spp. 19 (18.8%), *Klebsiella* spp. 13 (6.7%), and *Streptococcus* spp. 7 (3.6%). *Escherichia coli* isolates were highly resistant to Penicillin 100 (100%), Ceftriaxone (100%) and Tetracycline (80%) while *Klebsiella* spp. was found resistant to Amikacin (100%), Gentamicin (100%) and Ceftriaxone (100%). Similarly, *Staphylococcus* spp. obtained in the current study was found to be resistant to Ciprofloxacin (100%), Gentamicin (100%) and Amikacin (100%) while *Streptococcus* isolates were resistant to Tetracycline (100%), Penicillin (100%) and Chloramphenicol (100%). Susceptibility of the isolates were *E. coli* 100 (100%) for Chloramphenicol. Owners of dogs should refrain from using antibiotics indiscriminately. In conclusion, the study revealed the presence of dissimilar bacteria in the oral cavities of hunting dogs using cultural isolation, biochemical and antibiotics sensitivity tests in the study area.

Keywords: Antimicrobial susceptibility patterns, hunting dogs, oral cavity, oral microbiota, phenotypic.

INTRODUCTION

Infectious diseases in animals have been a significant concern for both veterinarians and public health professionals, with the oral cavity serving as a major reservoir for potentially pathogenic microorganisms (Kinnunen *et al.*, 2022). Amongst animals, hunting dogs play a crucial role in various communities, aiding in hunting activities and serving as loyal companions to their owners, and similarly, there is possible contamination of the hunted meat by these hunting dogs especially if the meat is not adequately processed for human consumption (Holland *et al.*, 2022; Santos *et al.*, 2022). Despite their vital roles, hunting dogs are exposed to diverse environmental and dietary factors, making them susceptible to various oral health issues and potential carriers of zoonotic pathogens (Rahman *et al.*, 2020; Keatts *et al.*, 2021; Mendoza &

Otranto, 2023). Understanding the oral microbiota composition and its antibiotic susceptibility pattern in these canines is of paramount importance for both animal welfare and public health implications.

Nigeria, with its diverse geographical regions and unique cultural practices, provides a promising setting for studying the oral microbiota of hunting dogs. Among the urban centers in Nigeria, Maiduguri stands as a significant hub for hunting activities, where hunting dogs are widely employed for various purposes (Mustapha *et al.*, 2016). However, to date, comprehensive studies on the oral microbiota of hunting dogs in this region are notably scarce.

Therefore, the current study aimed to bridge this critical knowledge gap by presenting an important step towards a deeper understanding of the oral aerobic bacterial microbiota among hunting dogs in Maiduguri, Nigeria. The insights

gained from the present study have the potential to influence policies related to animal health and public health, contributing to the overall welfare and safety of both human and canine populations in the region.

MATERIALS AND METHODS

STUDY AREA

This study was conducted in Maiduguri Metropolitan Council of Borno state Nigeria. It lies within the semi-arid zone of the North-Eastern part of Nigeria. Borno State is situated between Latitude 11° 46' 18"N – 11° 53' 21"N and Longitudes 13° 02' 23"E – 13° 14' 19"E. The town has a population of 1,197,497 people as of 2009 with Kanuri as the predominant tribe. The state occupies the greatest part of the Lake Chad Basin and shares borders with three West African countries via; the Republic of Chad to the Northeast, the Niger Republic to the North, and Cameroun Republic in the East (Google Earth, 2012).

COLLECTION OF SAMPLES

The current study was conducted from December 2021 to April 2022. One hundred and ninety-three (193) hunting dogs were sampled, and oral swab was collected using sterile swab sticks from the oral cavity of individual dogs presented at the hunting rendezvous in Maiduguri. Ten hunting dogs were randomly sampled weekly, while forty hunting dogs were sampled monthly and out of these numbers, seven samples were contaminated. Therefore, one hundred and ninety-three hunting dogs were used for the study. The oral swab samples obtained were labeled and transported in Stuart agar under cool chain to the Department of Veterinary Medicine Research laboratory, Faculty of Veterinary, University of Maiduguri.

PHENOTYPIC IDENTIFICATION OF BACTERIA

Cultural isolation and identification of bacteria were carried out as described by Cheesbrough (2010), each swab sample was inoculated onto 5% blood agar (Oxoid, UK) and MacConkey Lactose agar (Oxoid, UK). Inoculated plates were incubated aerobically at 37°C for 24-48 hours. After incubation, plates were examined for bacterial colonies. Isolates were identified by colonial morphology, microscopy (following Gram's staining) and biochemical characterization.

The biochemical tests applied were oxidase, catalase, starch hydrolysis, casein hydrolysis, indole tests, methyl-red and Voges-Proskauer test (MR-VP).

ANTIBIOTIC SUSCEPTIBILITY TEST

The antibiotic susceptibility testing of the isolates was carried out using the disc diffusion method according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2012). Briefly, well-isolated colonies were transferred into a test tube of brain heart infusion broth

(BHI) and incubated for 2 hours at 37°C, to obtain turbidity equivalent to 0.5 McFarland standards. With the help of a sterile cotton swab stick, the broth culture was then evenly spread by smearing over the surface of Mueller Hinton agar plates. The antibiotic discs were placed on the agar and gently pressed with sterile forceps to have a uniform close contact with the medium. The plates were then incubated at 37°C for 24 hours. The diameter of each zone of inhibition was measured from the edge to the edge of the disc. The interpretation of the measurement as sensitivity, intermediate, and resistance was made according to the clinical laboratory standards institute manual (CLSI, 2012). The antimicrobial agents tested were Tetracycline (30µg), Chloramphenicol (30 µg), Erythromycin (15 µg), Gentamicin (10µg), Penicillin (10µg), Vancomycin (30µg), Azithromycin (10µg) and Ceftriaxone (30µg), Ciprofloxacin (5µg), Amikacin (30µg) and Nalidixic acid (30µg).

DATA ANALYSIS

Descriptive statistics was used to present the data.

RESULTS

A total of 139 bacteria isolates were obtained from 193 oral swabs of hunting dogs. The bacteria were isolated from swabs irrespective of the individual. The most frequently identified isolates were *Escherichia coli* 100 (51.8%) followed by *Staphylococcus* spp. 19 (18.8%), *Klebsiella* spp. 13 (6.7%), and *Streptococcus* spp. 7 (3.6%) (Tables I). The result of the antimicrobial susceptibility test indicated that all the isolates were resistant to more than one antibiotic. *Escherichia coli* isolates were highly resistant to Penicillin 100 (100%), Ceftriaxone 100 (100%) and Tetracycline 80 (80%) while susceptible to Chloramphenicol 100 (100%) and Ciprofloxacin 87 (87%) as presented in Table II. *Staphylococcus* obtained in the current study was found to be resistant to Ciprofloxacin 19 (100%), Gentamicin 19 (100%), Amikacin 19 (100%) and maximum sensitivity was observed for Ceftriaxone 19 (100%) as shown in Table III. *Klebsiella* was found resistant to Amikacin 13 (100%), Gentamicin 13 (100%) and Ceftriaxone 13 (100%) while maximum susceptibility was found in Ciprofloxacin 13 (100%) as depicted in Table IV

Table I: Distribution of bacteria isolated from the oral cavity of hunting dogs (n = 193)

Bacterial Pathogen	No. of positive Isolates	Percentage %
<i>E. coli</i> spp.	100	51.8
<i>Staphylococcus</i> spp.	19	9.8
<i>Klebsiella</i> spp.	13	6.7
<i>Streptococcus</i> spp.	7	3.6
Total	139	71.9

Table II: Antibiotic susceptibility pattern of *E. coli* isolated from oral cavity of Hunting dogs (n=100)

Antibiotics	Resistance number of isolates (%)	Intermediate number of isolates (%)	Susceptibility number of isolates (%)
Amikacin	70(70)	9(9)	21(21)
Gentamicin	62(62)	16(16)	22(22)
Ciprofloxacin	10(10)	3(3)	87(87)
Chloramphenicol	0.0	0.0	100(100)
Azithromycin	5(5)	83(83)	12(12)
Penicillin	100(100)	0.0	0.0
Nalidixic acid	5(5)	79(79)	19(19)
Ceftriaxone	100(100)	0.0	0.0
Tetracycline	80(80)	5(3.5)	15(15)
Erythromycin	53(53)	47(47)	0.0

Table III: Antibiotic susceptibility pattern of *Staphylococcus* isolated from oral cavity of Hunting dogs (n=19)

Antibiotics	Resistance number of isolates (%)	Intermediate number of isolates (%)	Susceptibility number of isolates (%)
Amikacin	19(100)	0.0	0.0
Gentamicin	19(100)	0.0	0.0
Ciprofloxacin	19(100)	0.0	0.0
Chloramphenicol	0.0	19(100)	0.0
Azithromycin	5(50)	1(10)	4(40)
Penicillin	19(100)	0.0	0.0
Nalidixic acid	4(40)	3(30)	1(10)
Ceftriaxone	0.0	0.0	19(100)
Tetracycline	7(70)	2(20)	1(10)
Erythromycin	6(60)	0.0	4(40)

Streptococcus isolate was found sensitive to Gentamicin 7 (100%), Amikacin 7 (100%), Ciprofloxacin 7 (100%) and resistant to Tetracycline 7 (100%), Penicillin 7 (100%) and Chloramphenicol 7 (100%) as indicated in Table V.

DISCUSSION

Dogs' oral cavities may harbor bacteria that can contaminate bite wounds and potentially spread to humans through licking (Kisaka *et al.*, 2022; Tóth *et al.*, 2022). Previous research has identified drug-resistant micro-organisms of public health significance in both people and companion animals (Kaspar *et al.*, 2018).

Comparable to a study by Awoyomi and Ojo (2014), who reported 101 bacteria isolates in hunting dogs from Ogun State, the current study found 139 bacterial isolates from hunting dogs in the study area. The variation in the frequency of bacteria isolates could be attributed to differences in sample size. *Escherichia coli* was shown to be

the most prevalent bacterial species in the oral cavities of hunting dogs, according to the outcome of the present study. This may be due to hunting dogs' habitual indiscriminate consumption of all kinds of carcasses while on the hunt. Ofukwu *et al.* (2008) and Bata *et al.* (2020) also found similar results, although Awoyomi and Ojo (2014) identified *Bacillus* species to be the most frequently encountered bacteria. The findings of the present study are in accordance with those of Almansa Ruiz *et al.* (2018), Razali *et al.* (2020), and Adzitey *et al.* (2022), who reported *Escherichia coli* from the oral cavity of dogs in previous investigations. However, none of these authors has explained the high isolation rate of *Escherichia coli* bacteria in dogs' oral cavities. The species of *Staphylococcus*, *Klebsiella*, and *Streptococcus* found in this investigation are like those cited by Bata *et al.* (2020). In contrast to earlier reports by Awoyomi & Ojo (2014) and Bata *et al.* (2020), these isolates were more frequently found in the current study. Perhaps, the discrepancy is due to environmental factors such as contaminated milieu. The results of the present study are consistent with those of Misic *et al.* (2015) and Razali *et al.* (2020), who reported the presence of *Staphylococcus* and *Streptococcus* species in dogs' oral cavities. *Staphylococcus* species have also been reported in dogs in other investigations by Paul *et al.* (2014), Bean and Wigmore (2016), and Razali *et al.* (2022). These

bacteria were also reported as the second most common species isolated from dog bite wounds (Abrahamian and Goldstein, 2011). Given that hunting dogs frequently scavenge and are exposed to various bacterial organisms during their feeding and hunting activities, it is logical to hypothesize those environmental factors, diet, and living conditions may affect the types and frequencies of bacteria in a dog's oral cavity (Abrahamian & Goldstein, 2011). According to Abrahamian and Goldstein (2011), some bacteria might be rare and not frequently encountered in the oral microbiota. All the dogs recruited for the present study were trained to hunt, exposing them to a variety of bacterial species from the environment as well as contaminated food and prey. This emphasizes how crucial it is to take these factors into account when figuring out what kind of bacteria are present in canine oral cavities (Awoyomi & Ojo, 2014).

The current study on antimicrobial susceptibility test showed that certain bacterial isolates were resistant to multiple antibiotics. Complete resistance to Penicillin, Ceftriaxone, and Tetracycline was seen in *Escherichia coli* isolates, correlating with VasIU *et al.* (2022) discovery of *Escherichia coli* resistance to Penicillin. The present study suggests a serious risk to the ability of these antibiotics to effectively treat wounds caused by dog bites and aligns with a similar concern raised by Awoyomi and Ojo (2014). However, *Escherichia coli* isolates displayed high susceptibility to Chloramphenicol and Ciprofloxacin, suggesting that these antibiotics could be the preferred choices for effectively treating dog bite wounds or infections caused by pathogenic strains of *Escherichia coli*. This finding is consistent with a report by Bata *et al.* (2020).

In open wounds in humans and animals, *Staphylococcus* species have been identified as the main cause of suppurative infections, abscesses, and septicaemia. The *Staphylococcus* isolates in this investigation displayed multi-drug resistance to Ciprofloxacin, Gentamicin, and Amikacin, which suggests the possibility that these antibiotics might not be able to successfully treat infections brought on by dog bites or saliva. The finding corresponds to that published by Gomez-Beltran *et al.* (2020), who reported Gentamycin, Ciprofloxacin, and Tetracycline resistance in *Staphylococcus* species. However, VasIU *et al.* (2022) findings, which showed *Staphylococcus* species resistant to Erythromycin, Penicillin, and Ceftriaxone, contrasts with the results of the current study. Although MRSA infections cause a high rate of morbidity and mortality in humans, its global spread poses

Table IV: Antibiotic susceptibility pattern of *Klebsiella* isolated from oral cavity of Hunting dogs (n =13)

Antibiotics	Resistance number of isolates (%)	Intermediate number of isolates (%)	Susceptibility number of isolates (%)
Amikacin	13(100)	0.0	0.0
Gentamicin	13(100)	0.0	0.0
Ciprofloxacin	0.0	0.0	13(100)
Chloramphenicol	4(30.76)	0.0	9(69.23)
Azithromycin	5(38.46)	7(53.84)	1(7.69)
Penicillin	7(53.84)	6(46.15)	0.0
Nalidixic acid	3(23.07)	9(69.23)	1(1.69)
Ceftriaxone	13(100)	0.0	0.0
Tetracycline	5(38.46)	5(38.46)	3(23.07)
Erythromycin	2(15.38)	11(84.61)	0.0

Table V: Antibiotic susceptibility pattern of *Streptococci* isolated from oral cavity of Hunting dogs (n =7)

Antibiotics	Resistance number of isolates (%)	Intermediate number of isolates (%)	Susceptibility number of isolates (%)
Amikacin	0.0	.0	7(100)
Gentamicin	0.0	1(20)	4(80)
Ciprofloxacin	0.0	0.0	7(100)
Chloramphenicol	7(100)	0.0	0.0
Azithromycin	1(20)	1(20)	3(60)
Penicillin	7(100)	0.0	0.0
Nalidixic acid	2(40)	1(20)	2(40)
Ceftriaxone	2(40)	0.0	3(60)
Tetracycline	7(100)	0.0	0.0
Erythromycin	2(40)	2(40)	1(20)

a threat (Boucher and Corey, 2008). Dogs have been identified as reservoirs of MRSA transmissible to humans. Studies have shown that humans and their pets harbor similar MRSA strains (Baptiste *et al.*, 2005; Mustapha *et al.*, 2016). Antibiotic-resistant Staphylococcal infections in humans have been documented (Pottumarthy *et al.*, 2004). *Escherichia coli* and *Staphylococcus* species are still regarded as antibiotic-resistant bacteria even though the isolates showed the greatest sensitivity to ceftriaxone, demonstrating viable treatment choices (Cheung, 2017).

The results of the current investigation showed that *Klebsiella* species exhibited resistance to Ceftriaxone, Gentamicin, and Amikacin. Nevertheless, Ciprofloxacin's high susceptibility suggests a higher chance of treatment success, making it a potentially effective antibiotic for treating infections brought on by *Klebsiella* species isolated from dogs' oral cavities in the research area or dog bite wounds. This result is consistent with the findings of Bata *et al.* (2020), who discovered that *Klebsiella* species were very susceptible to Ciprofloxacin.

In contrast to Tetracycline, Penicillin, and Chloramphenicol, *Streptococcus* isolates were shown to be resistant to Gentamicin, Amikacin, and Ciprofloxacin. This finding backs up a related study by Awoyomi and Ojo (2014), who discovered that *Streptococcus* isolates were susceptible to Ciprofloxacin but resistant to Penicillin and Chloramphenicol. Contrary to Awoyomi and Ojo (2014) reports, the current investigation reveals that *Streptococcus* isolates are sensitive to Gentamicin.

CONCLUSIONS

The current investigation confirmed the existence of

resistant bacteria in the oral cavity of hunting dogs in Maiduguri. According to the current study, *E. coli* had the highest rate of infection. Most of the isolates showed two or more antibiotic resistances. This shows that multi-drug resistant bacteria are present in the oral cavity of hunting dogs and may spread to people through licks or infected bite wounds.

As a result, the present study recommends responsible dog ownership. Since only dogs that have been managed extensively have been shown to be responsible for spreading infection through bites, dogs should be controlled thoroughly. Drug usage that is inappropriate and indiscriminate ought to be avoided. However, given that bacterial isolation and antibacterial sensitivity testing are not widespread practices, preventive antibiotic treatment for high-risk wounds may not be without difficulty and may have serious consequences. Before starting treatment, microorganisms at bite wound sites should be isolated and tested for antibiotic sensitivity.

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CONFLICT OF INTEREST

Authors declare no competing interest in publishing this research work.

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